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Chemical Constituents of Peppers (*Piper* spp.) and Application to Food Preservation: Naturally Occurring Antioxidative Compounds

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In a structure analysis of the compounds of the genus *Piper* (Family Piperaceae), we identified five phenolic amides from *Piper nigrum*, seven compounds from *P. retrofractum*, and two compounds from *P. baccatum*. All the phenolic amides possess significant anitoxidant activities that are more effective than the naturally occurring antioxidant, α-tocopherol. One amide, feruperine, has antioxidant activity as high as the synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Naturally occurring antioxidants, therefore, may surpass BHA and BHT in their ability to inactivate mutagens in food.

Introduction

Much research has involved finding ways to prevent or to delay deterioration in foods. Recently, our research (*1,2*) has focused on developing safe and effective compounds from natural sources (especially from edible plants) that will prolong the storage life of food.

We studied the genus *Piper* of the Family Piperaceae, to identify the structure of the compounds in its species that give organoleptic (*3–5*), medicinal (*6–9*), and insecticidal (*10–13*) properties. Many species, among the 700 in this genus, are used not only as spices but also as folk medicines (*14*). This paper reveals the structure of the compounds and their resulting properties for *Piper nigrum* L., *P. retrofractum* Vahl., and *P. baccatum* Blume.

Structural Determination of Constituents

Powdered dry fruits of white pepper (*P. nigrum*) were extracted with methylene chloride and fractionated as shown in Figure 1. The fractions determined were neutral (83.80%), weakly acidic (1.72%), strongly acidic (0.55%), and basic (0.22%). We found piperine [1] (a pungent principle) and more than 40 constituents isolated from *P. nigrum* to be neutral compounds. The weakly acidic fraction showed significant anitoxidative activity, and we subjected it to column chromatography on silica gel, using a solution of CH_2Cl_2 and MeOH as an eluant. The major compound [2a], purified upon recrystallization from hot chloroform (mp 144.0–144.5°C), produced colorless needles.

Mass spectral and elemental analyses of [2a] indicated the molecular formula $C_{18}H_{19}NO_4$.

Three absorption bands at 3500, 3360, and 3240 cm^{-1} in the infrared (IR) spectrum showed the presence of hydroxy or amino groups. The absorptions at 1645, 1615, and 980 cm^{-1} represented a *trans*-conjugated amide carbonyl group.

The λ_{max} at 295 and 321 nm in the ultraviolet (UV) spectrum indicated the presence of feruloyl moiety in the molecule, as shown in the spectrum of ferulic acid (λ_{max} 294 and 320 nm). This was also confirmed by the base peak at *m/z* 177 in the mass spectral (MS) data.

Further evidence for the structural characteristics was obtained from nuclear magnetic resonance (NMR) spectroscopy. One methoxy group was observed at δ 3.88, and a typical set of two doublets at δ 5.47 and δ 7.47 (both J = 16 Hz) was assigned to *trans*-α and *trans*-β-protons conjugated to the carbonyl group, respectively. A triplet (2H) centered at δ 2.75, (J = 7 Hz) and

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